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FREE AMINO ACIDS OPTIMIZE SYMBIOTIC ALGAE METABOLISM

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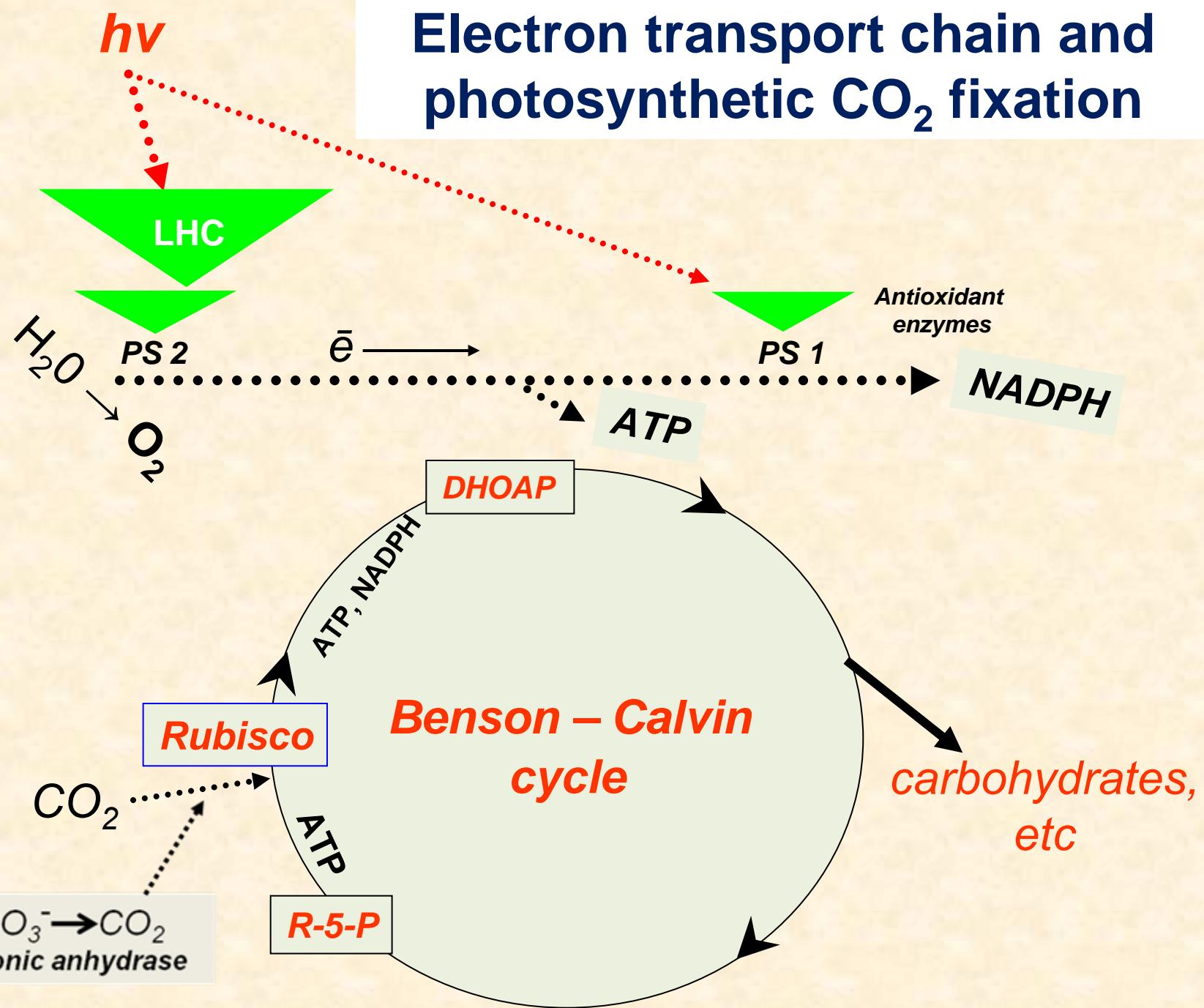
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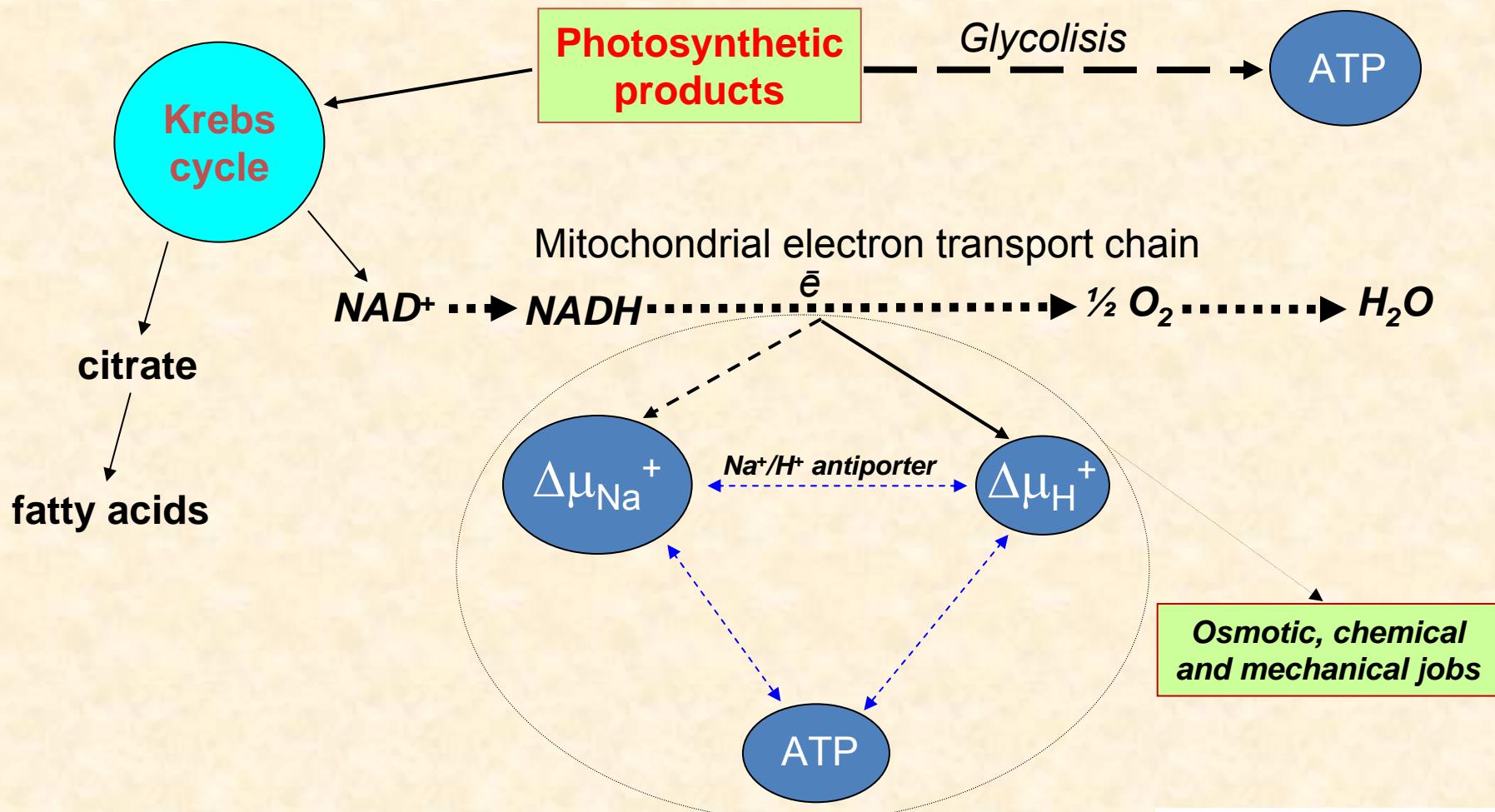
CONTENTS

- “Host Factor” and his influence on some algae functions
(HF optimizes environments for algae such that they fully realize their physiological potential)
- A hypothetical mechanism by which HFs influence on algae metabolism
- Practical applications of these knowledge
(Maximizing algae productivity will require conditions simulating HF such as found in wastewater)

Electron transport chain and photosynthetic CO_2 fixation

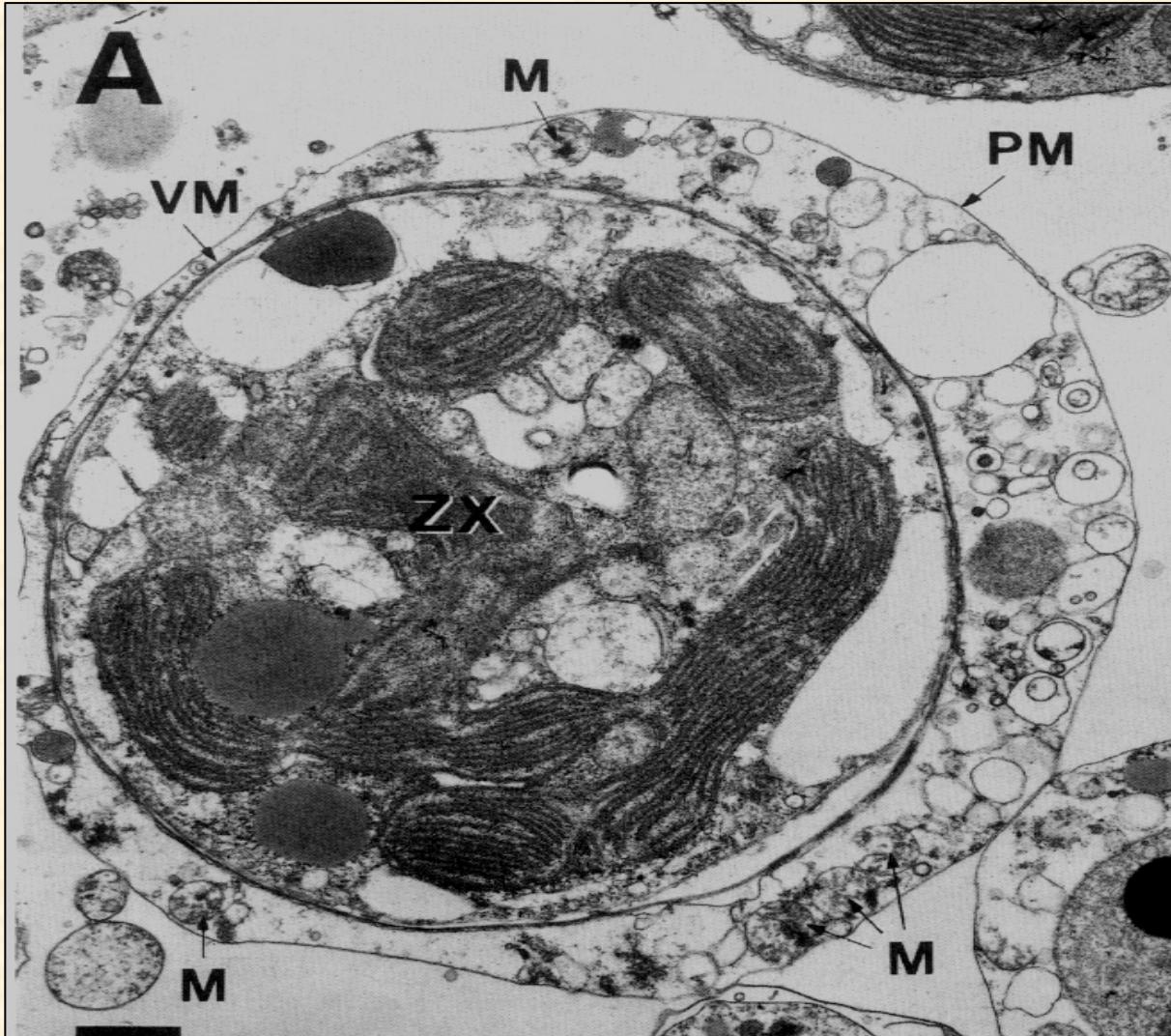


Respiration



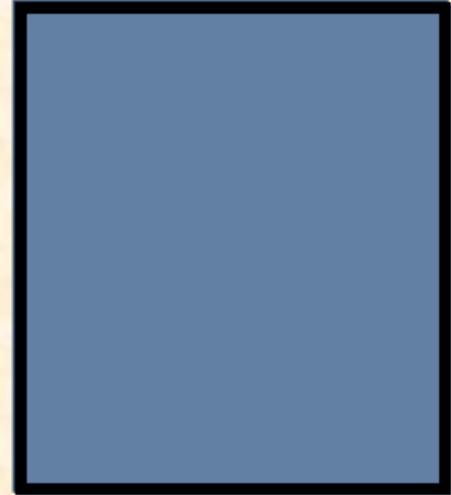
Modified from Skulachev, 1997;
Semikhatova and Chirkova, 2001;
Biel and Yensen 2004; 2005

Host cell and symbiotic alga inside



Symbiotic alga (zooxanthellae) of *Pocillopora damicornis*

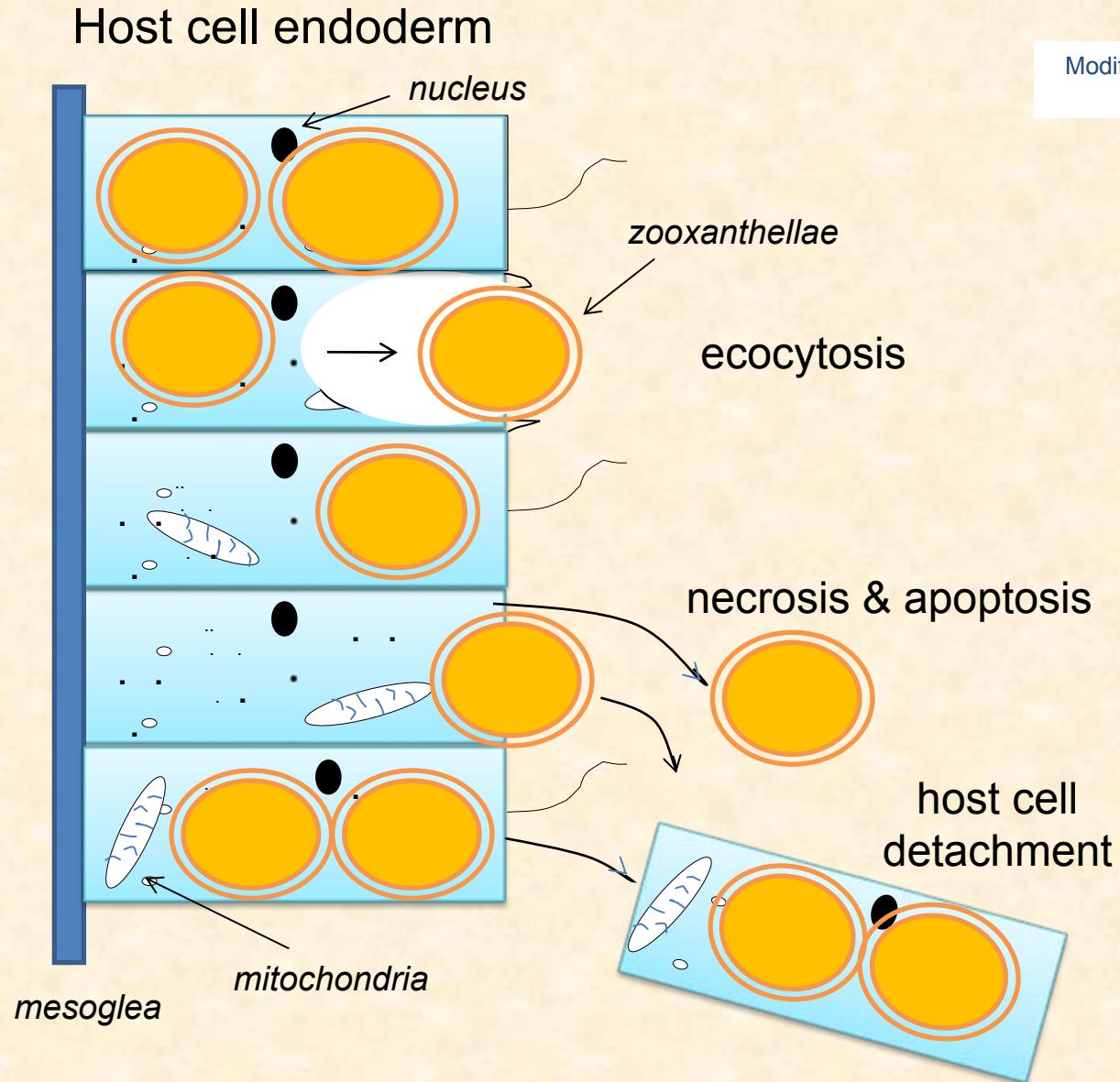
PM – host cell plasma membrane
ZX – zooxanthellae (alga)
VM – vacuolar membrane
M – mitochondria
(bar = 1 μ m)



Professor Leonard Muscatine (UCLA)

Glycerol excretion by symbiotic algae from coral and *Tridacna* and its control by the host. Science 1967 **156**: 516-519

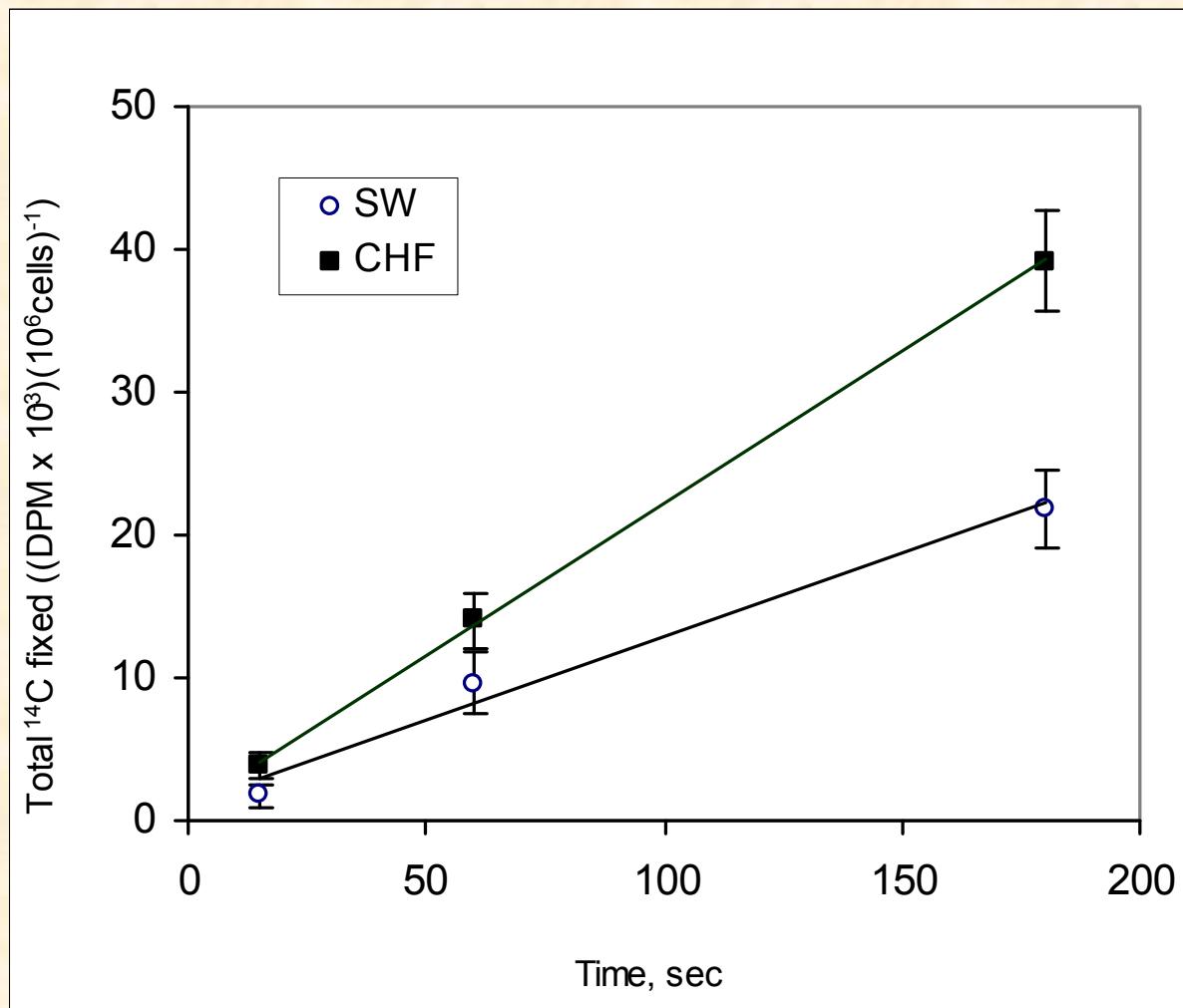
Cnidarians' endoderm and symbiotic algae



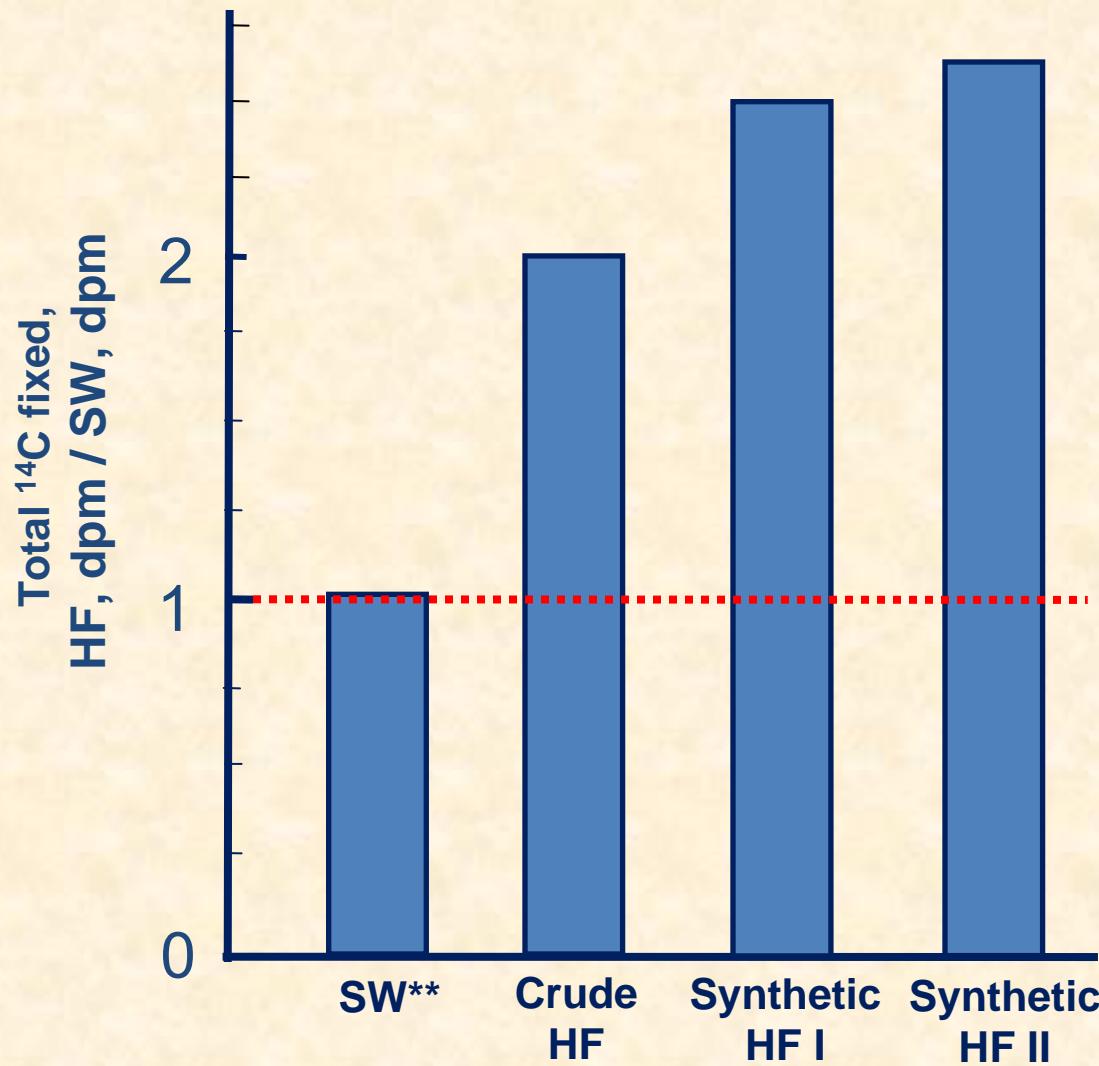
Free amino acid pools of two colonies of *Pocillopora damicornis*

Amino acid	Synthetic HF I	Synthetic HF II
	[mM]	
Aspartic acid	1.80	0.68
Glutamic acid	21.80	8.50
β -Glutamic acid	1.20	2.41
Serine	1.96	1.65
Histidine	1.84	0.00
Glycine	11.27	2.98
Arginine	2.25	1.96
Taurine	3.85	2.41
Alanine	12.89	7.93
Tyrosine	1.32	0.61
Methionine	3.08	0.37
Valine	4.73	7.12
Phenylalanine	3.17	1.47
Isoleucine	2.26	3.56
Leucine	2.64	2.22
Asparagine	0.00	1.28
Total	76.06	45.15

Photosynthetic CO_2 fixation by *A. pulchella* algae incubated in Sea Water and Crude Host Factor



Effect of “Host Factor” on CO₂ photoassimilation by symbiotic algae*



**Symbiodinium pulchrorum* isolated from *A. pulchella*

**Sea water

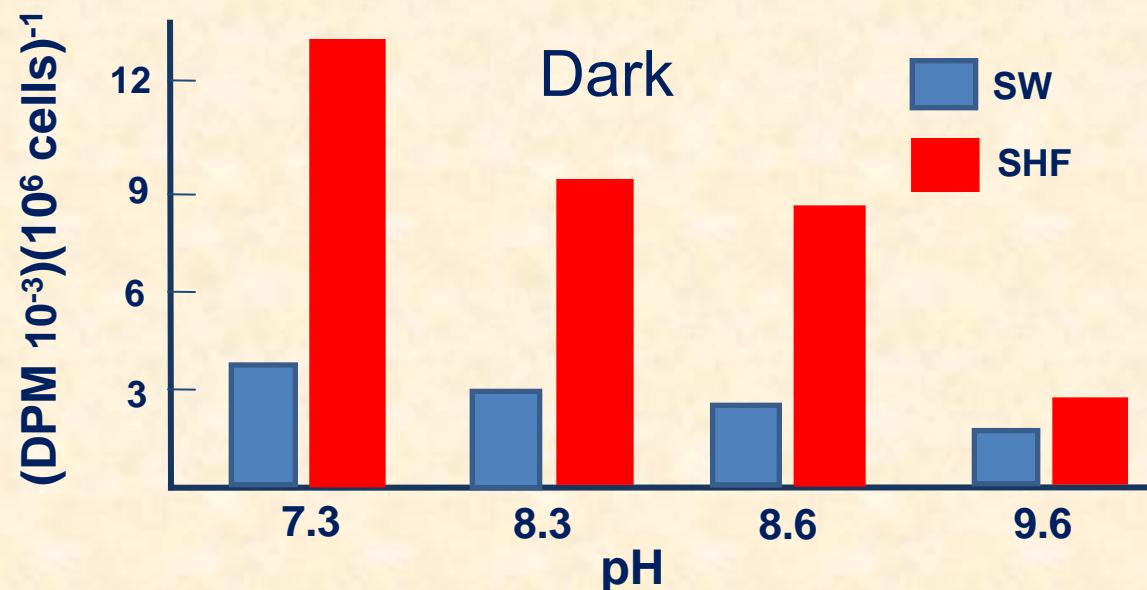
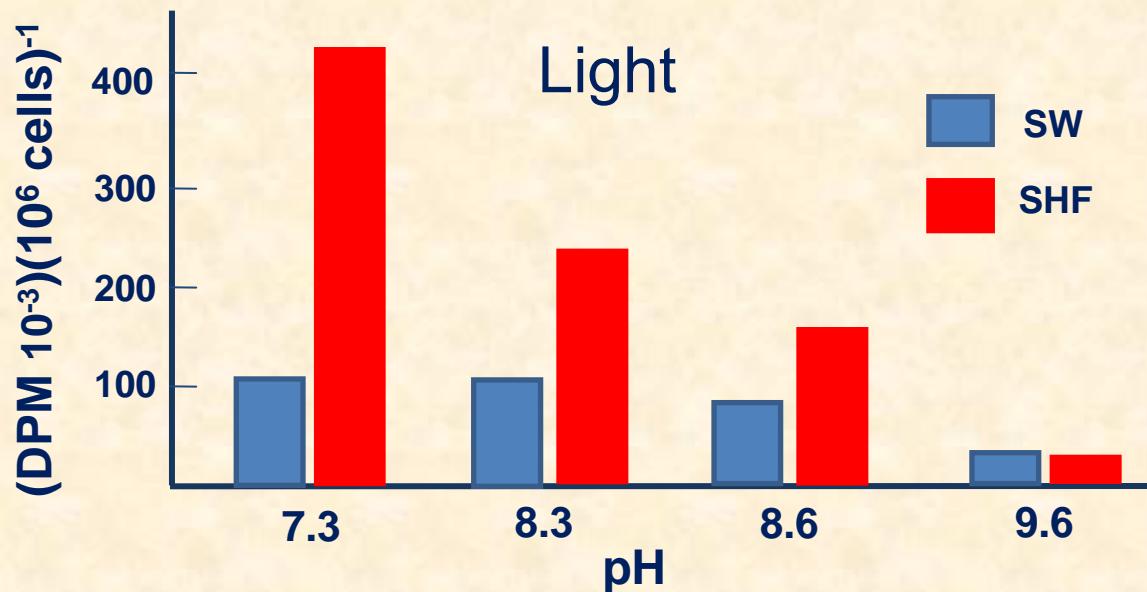
Modified from Gate et al 1995
Proc. Natl. Acad. Sci. USA 92:7430-7434

Photosynthesis and dark CO₂ fixation in *A. pulchella* algae in the presence of DCMU¹

Treatment	Total ¹⁴ C fixed ((DPM 10 ⁻³)(10 ⁶ cells) ⁻¹)	
	Light	Dark
SW	104	4
SHF	241	<u>11</u>
SW + DCMU ²	5	5
SHF + DCMU	<u>11</u>	<u>10</u>

¹DCMU – 3-(3,4-dichlorophenyl-1,1-dimethylurea) – inhibitor of photosynthesis

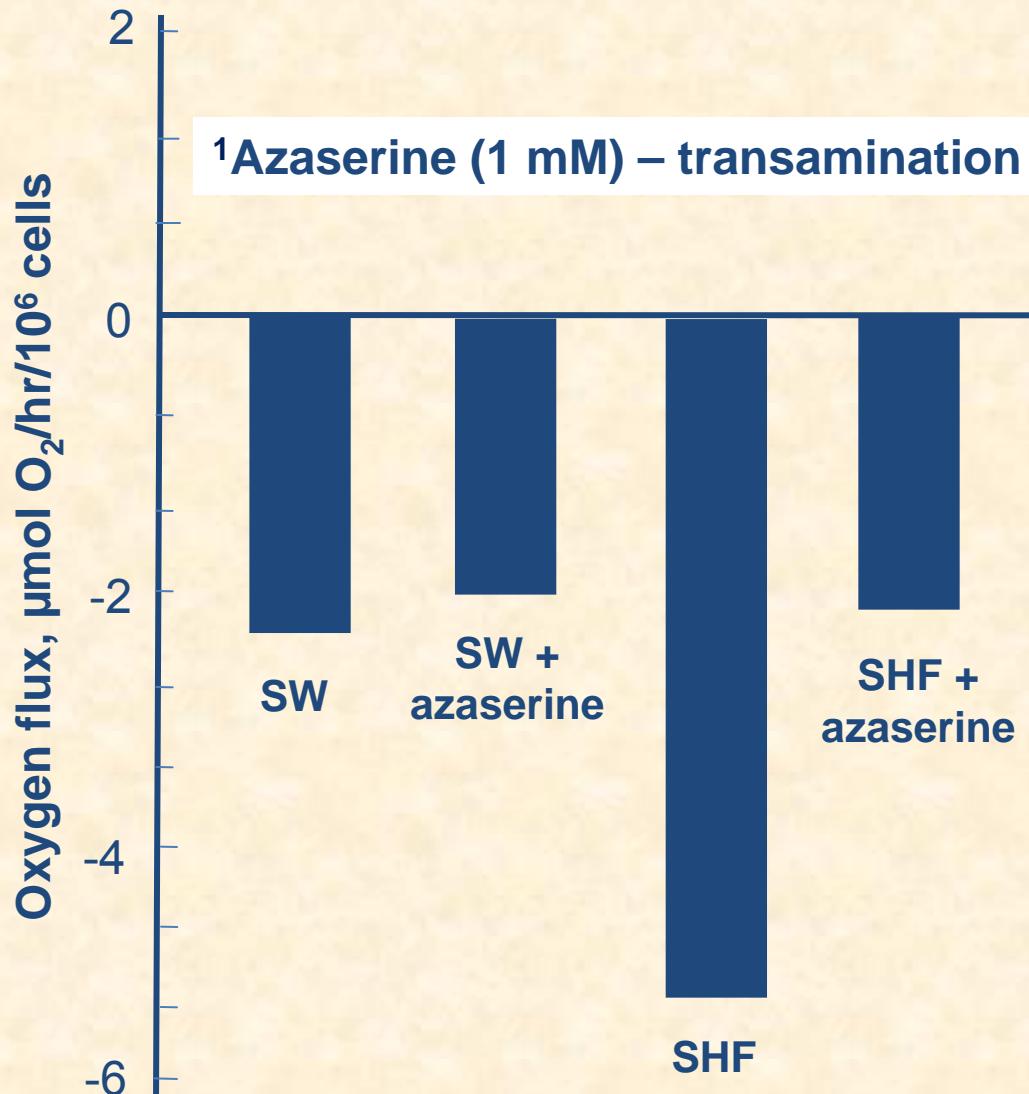
Photosynthesis and dark CO₂ fixation in *A. pulchella* algae at different pH



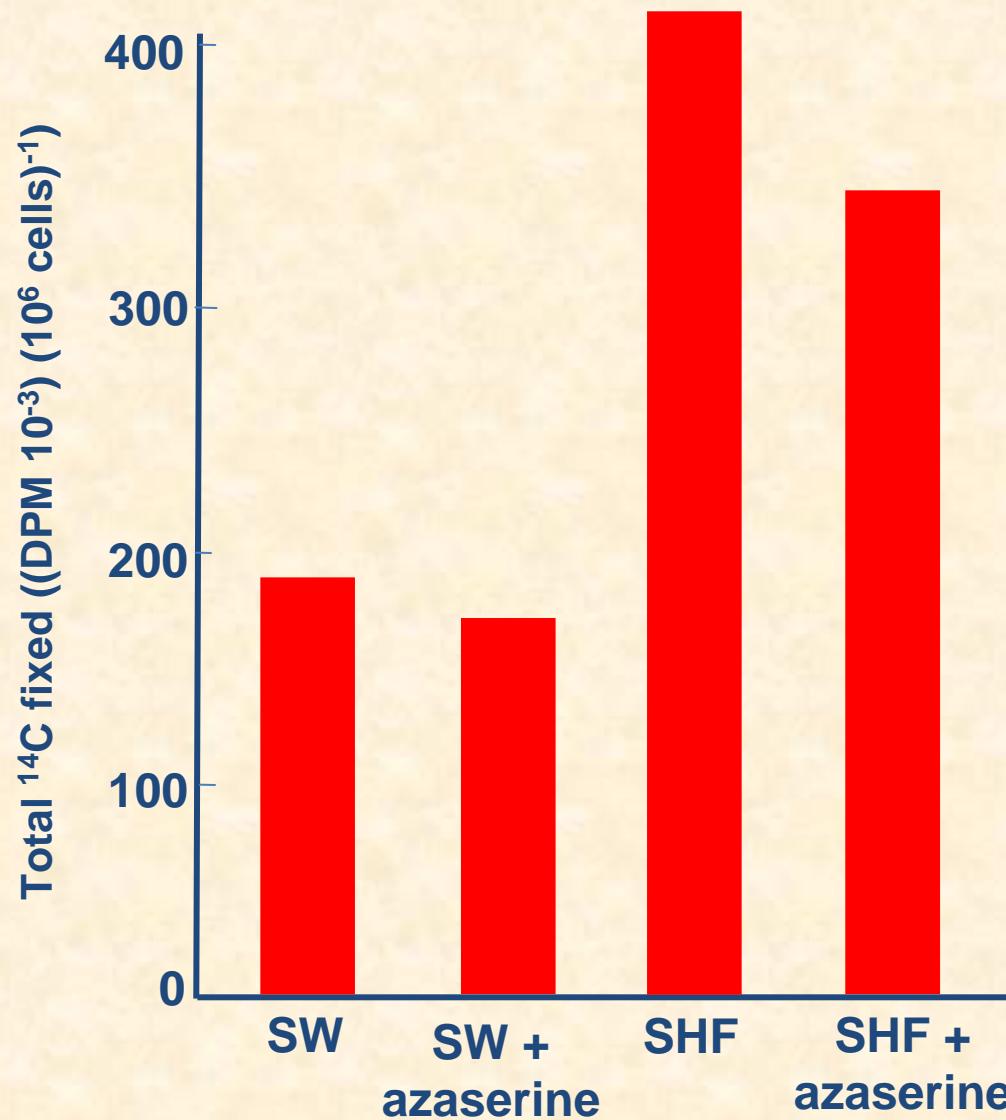
Enzymes of heterotrophic carboxylation:

- Phosphoenol pyruvate (PEP) carboxylase
 - PEP carboxykinase
 - Pyruvate carboxylase
 - **Acetyl CoA carboxylase**

Effect of azaserine¹ on oxygen flux in symbiotic *A. pulchella* algae

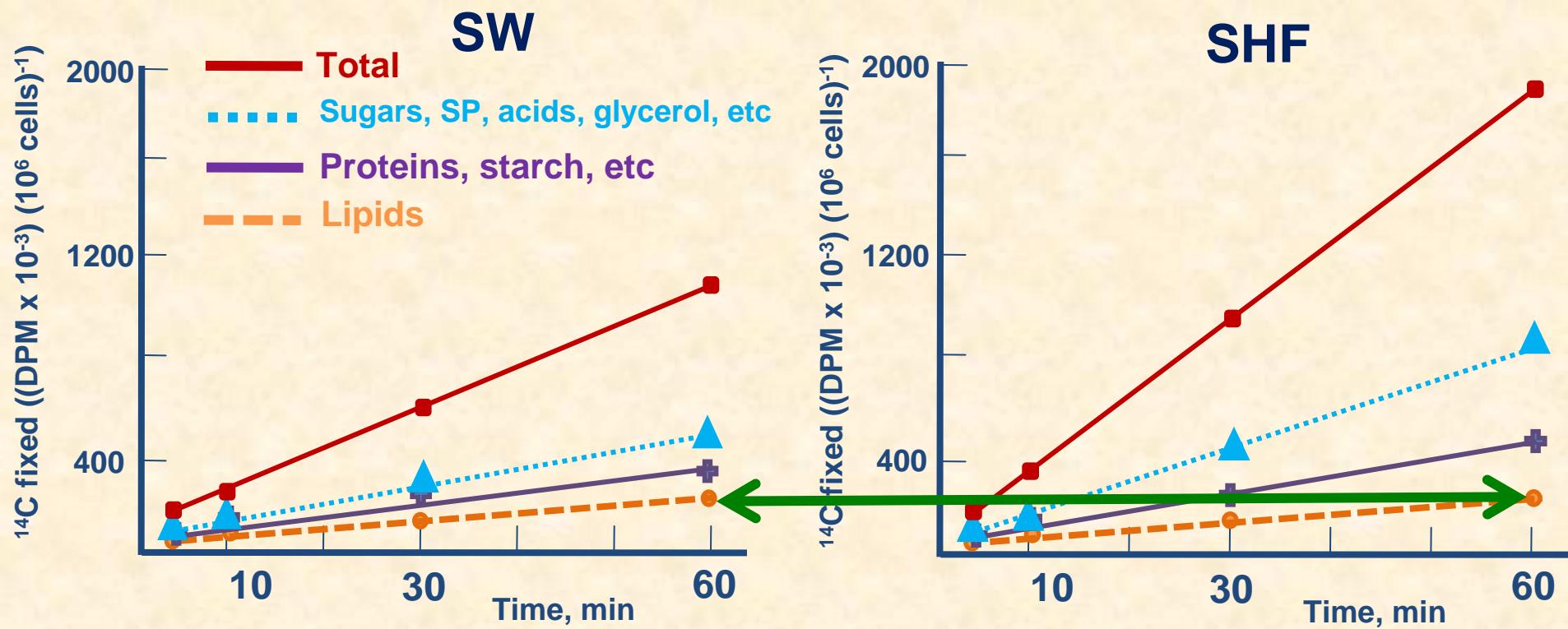


Effect of azaserine¹ on photosynthesis in symbiotic *A. pulchella* algae

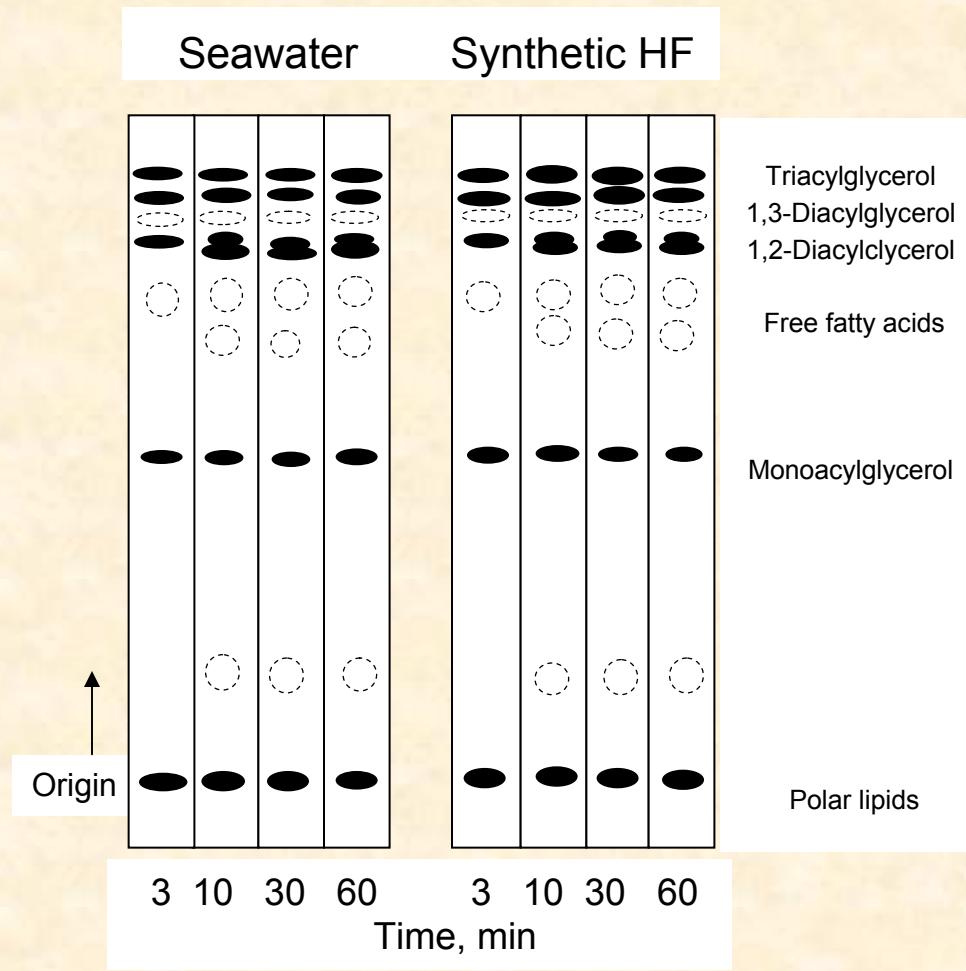


¹Azaserine – transamination inhibitor

Total CO₂ photoassimilation and ¹⁴C partitioning in *A. pulchella* algae vs. of time

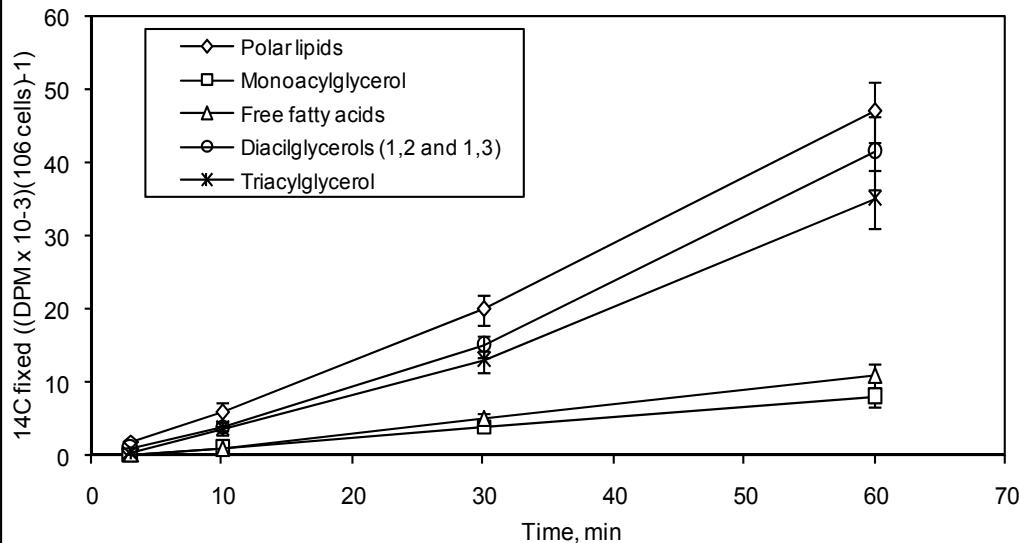


Lipids extraction:
methanol – chloroform (2:1; v:v)



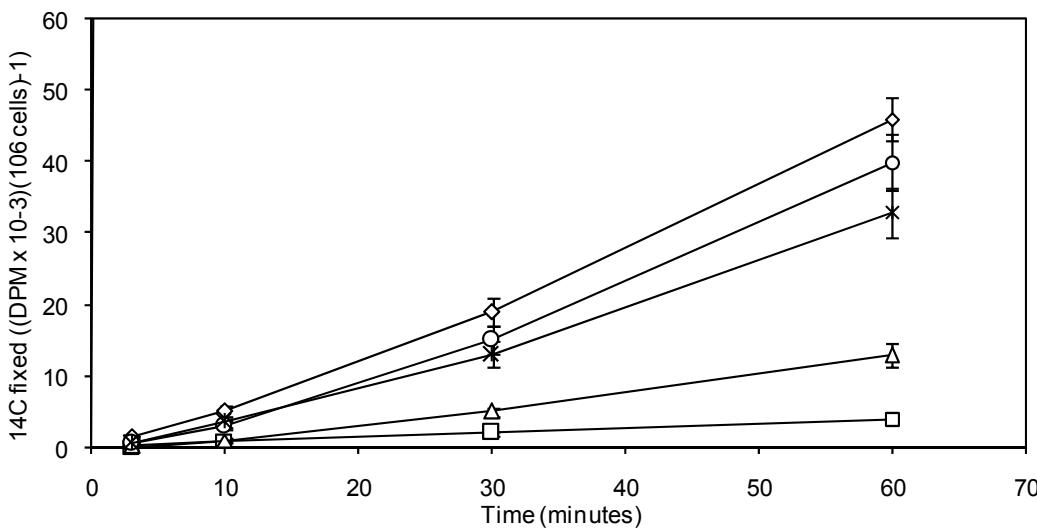
Neutral ^{14}C -lipids separated by one-dimensional TLC using PE silica gel/UV flexible plates and petroleum ether : acetone : chloroform as the solvents

Seawater

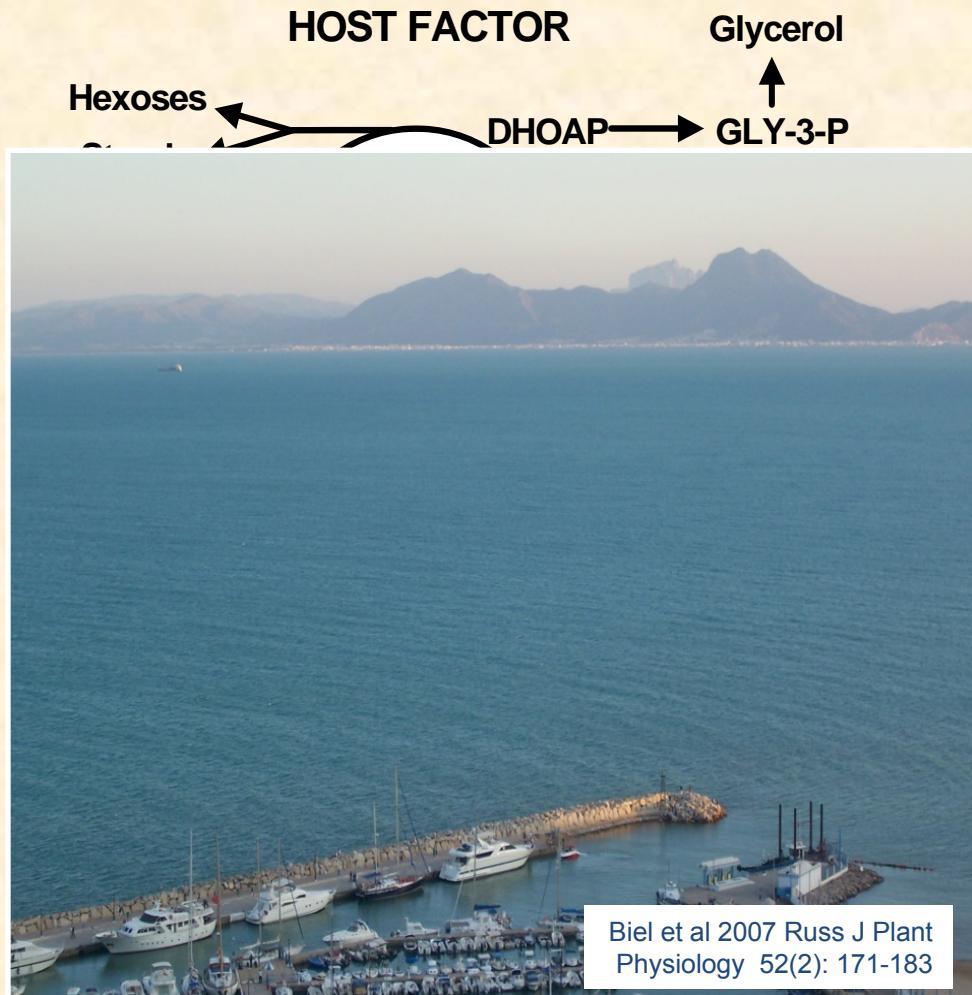
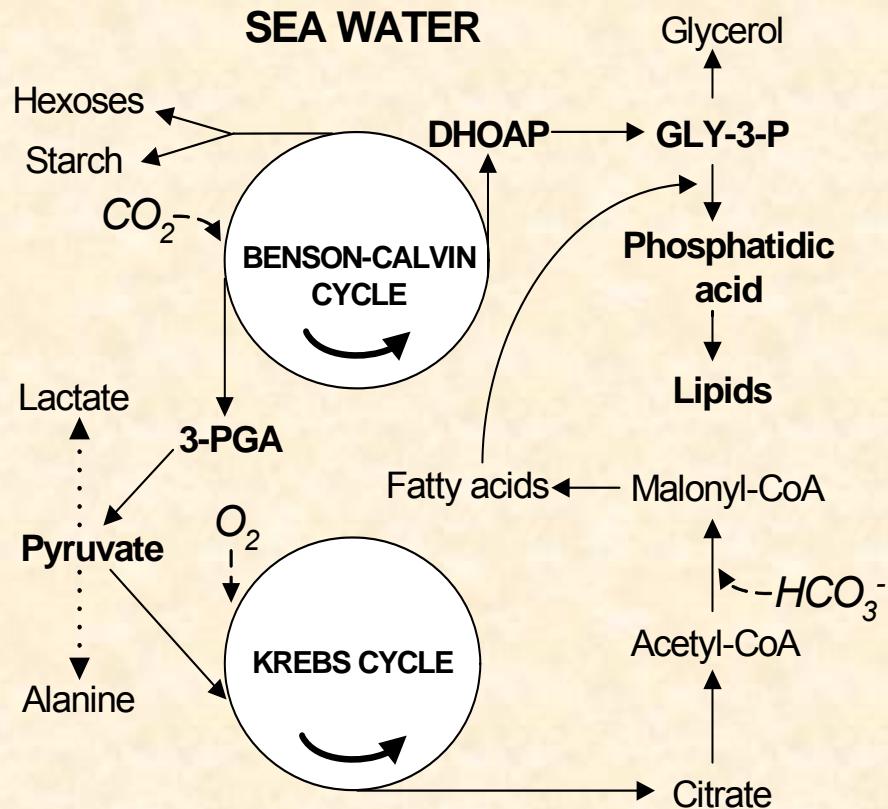


Incorporation of ^{14}C into different neutral lipids by *A. pulchella* algae vs. of time

Synthetic Host Factor



Influence of “Host Factor” on photosynthesis, respiration, and the first step of lipid creation in symbiotic and non-symbiotic algae

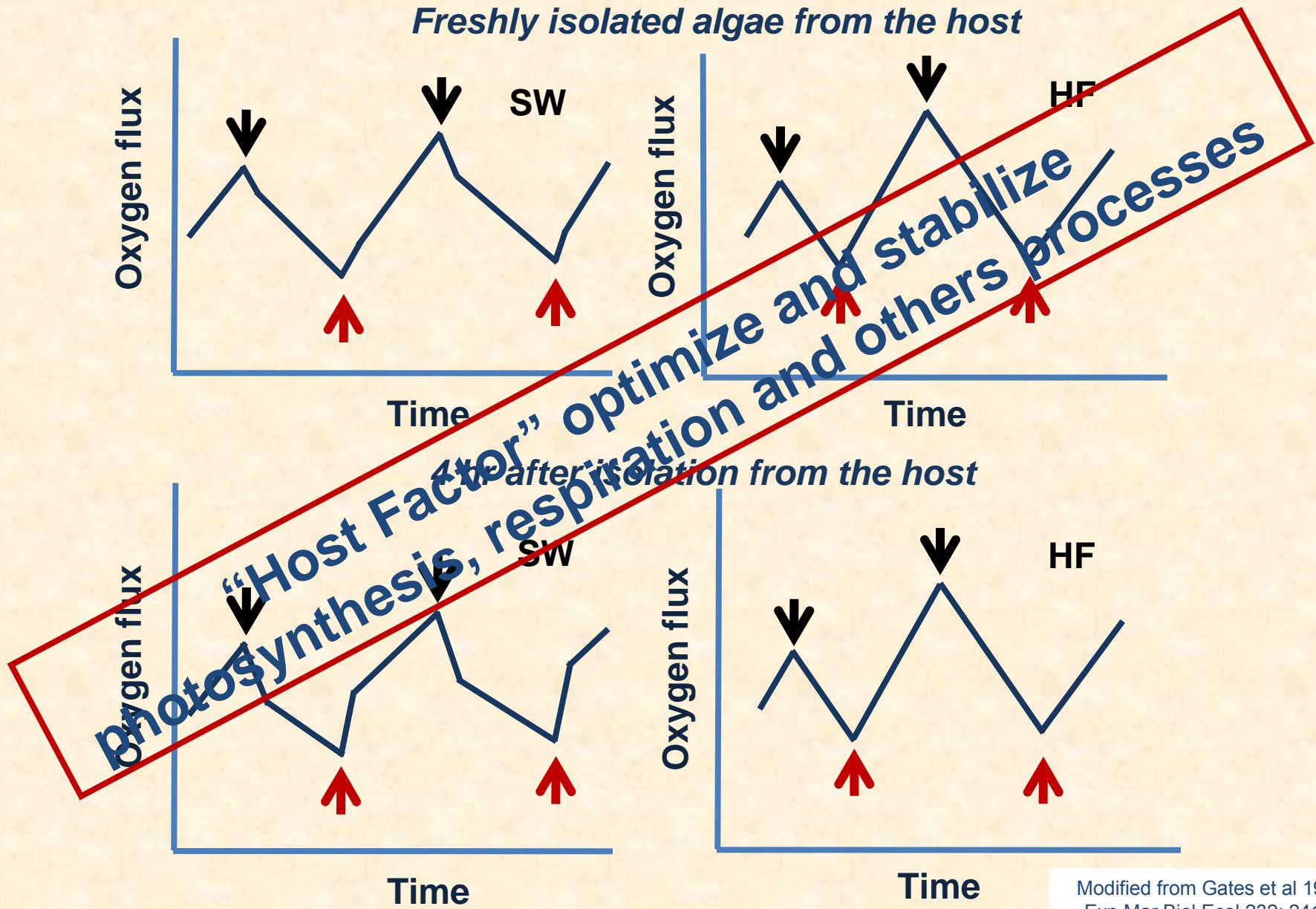


Biel et al 2007 Russ J Plant Physiology 52(2): 171-183

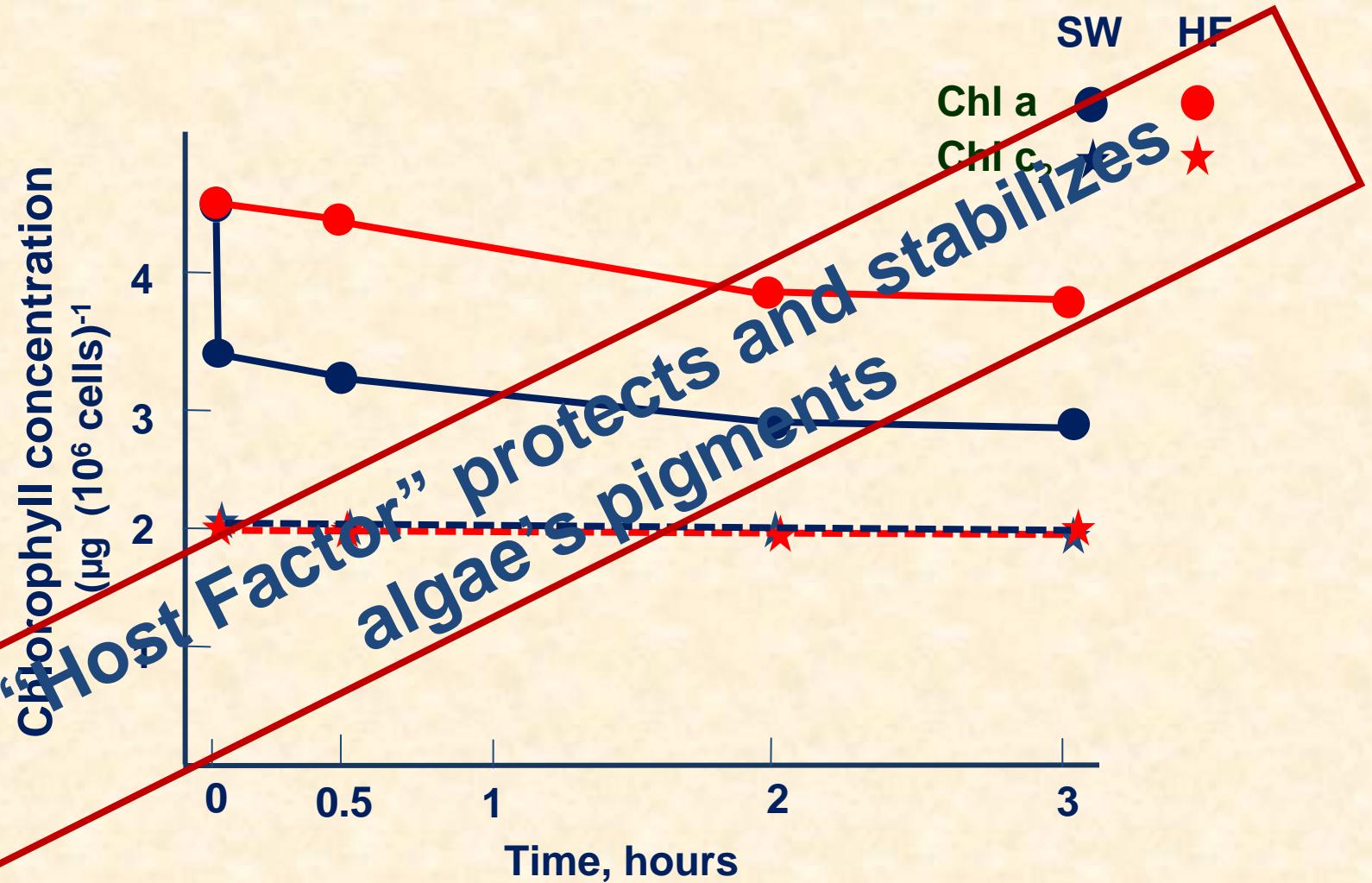
A scenic landscape photograph featuring a calm lake in the foreground. Behind the lake is a grassy hillside with patches of brown and green. In the background, there are several majestic mountains with rugged peaks. The sky above is a clear blue with some wispy, white clouds.

“Host Factor” Applications

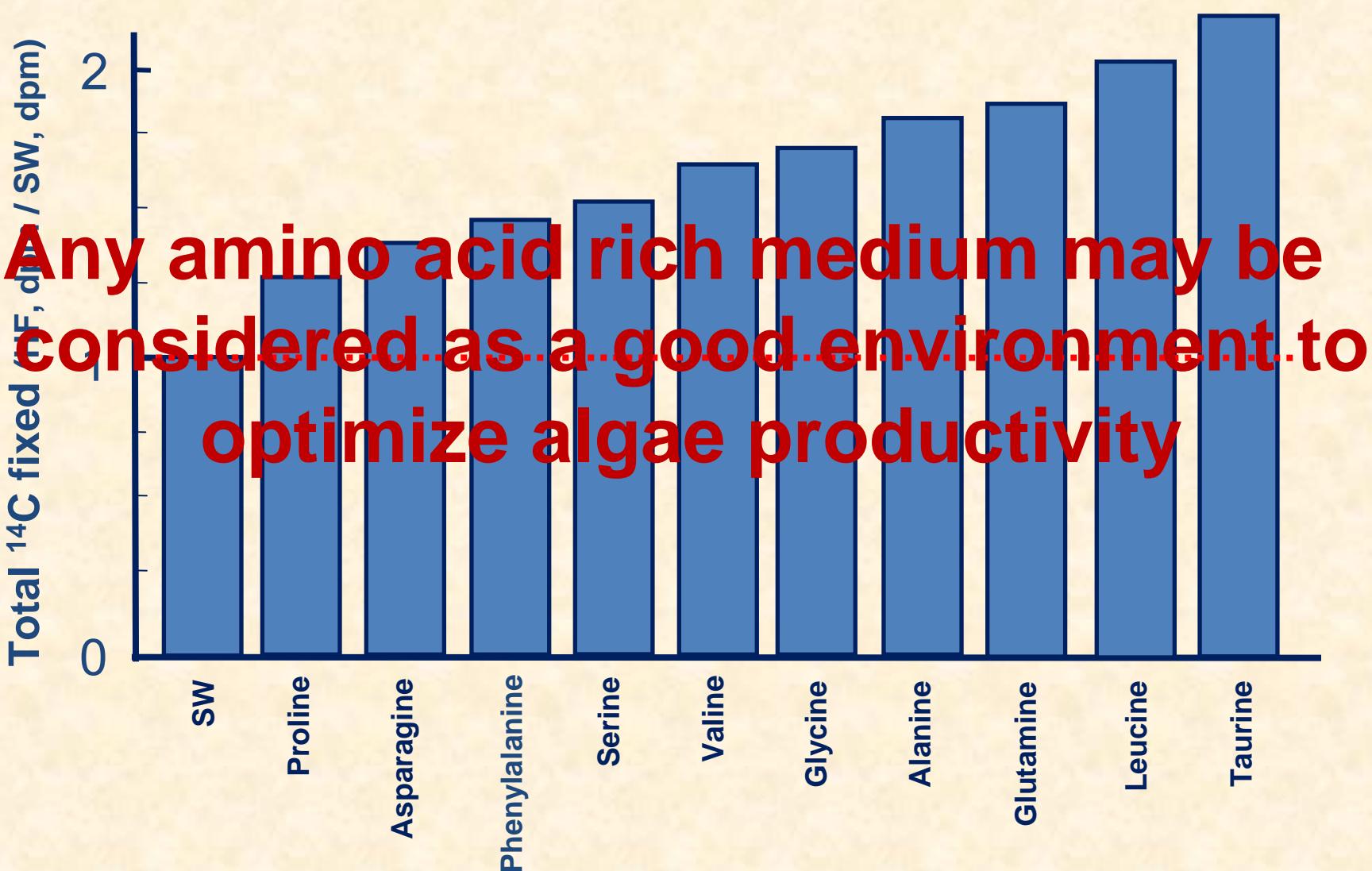
Oxygen flux (light/dark) of *A. pulchella* algae



Effect of isolation and maintenance of *A. pulchella* algae in SW and HF on chlorophylls



Effect of amino acids* on CO₂ photoassimilation by symbiotic algae



*50 mM - each amino acid diluted in distilled water;
salinity - 33 parts per thousand, pH 8.3

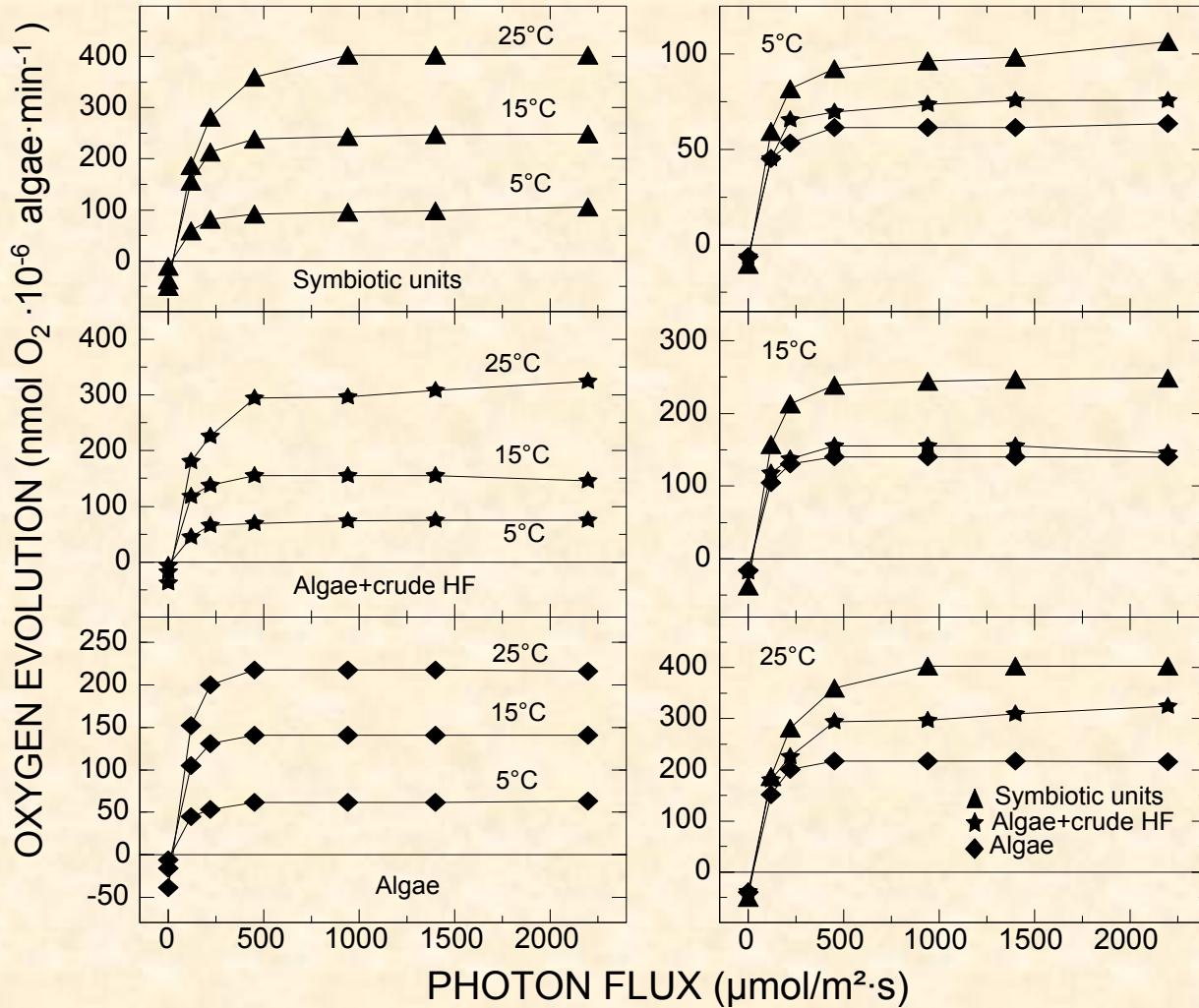
Modified from Gate et al 1995
Proc. Natl. Acad. Sci. USA 92:7430-7434

Spongilla lacustris

Swastika Lake, 3200 m, WY, USA

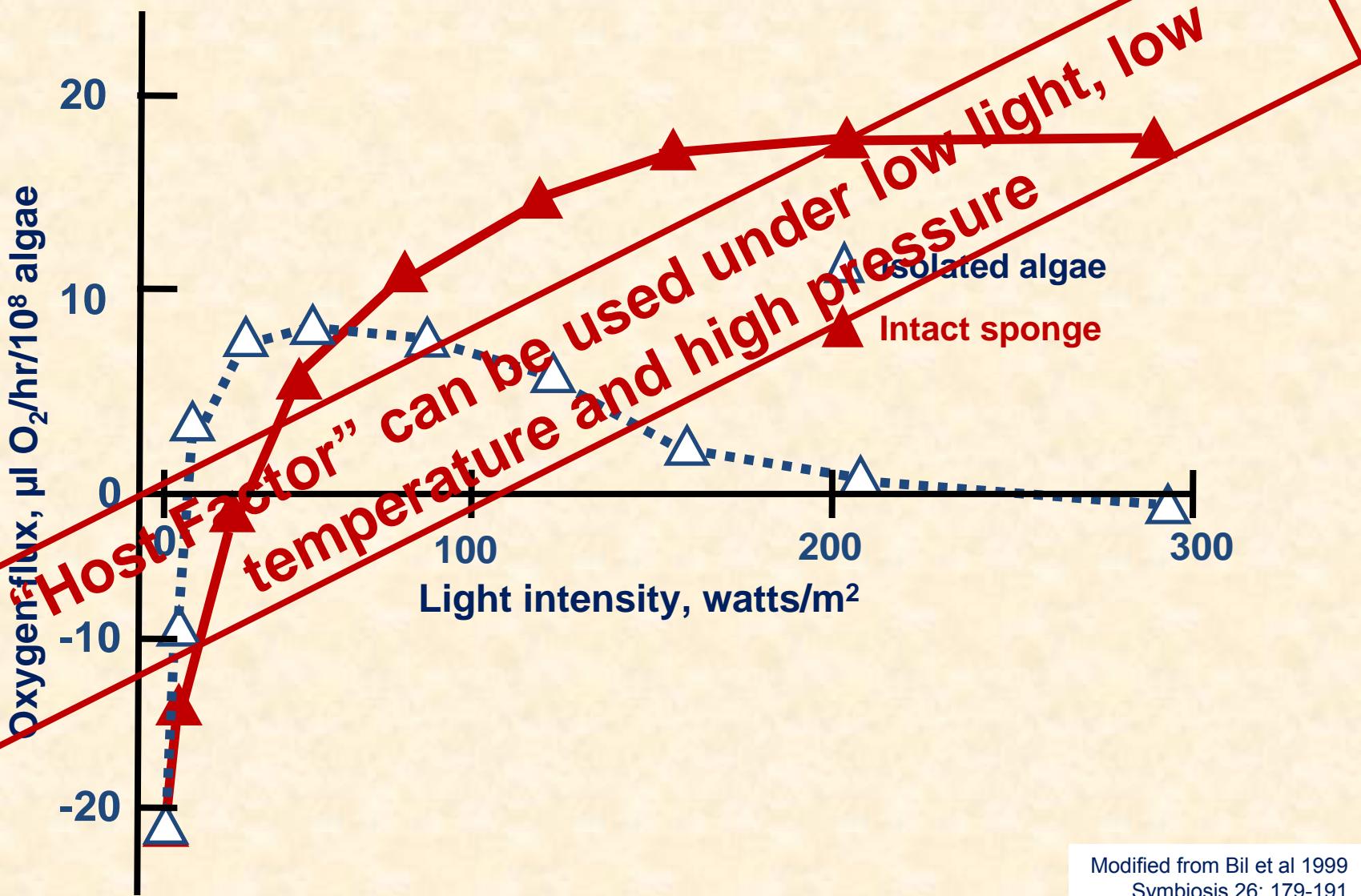
“Host Factor” can be used under high light, low temperature and low pressure

Light curves of *Spongilla lacustris* vs. of temperature



- Rates of O₂ evolution were highest in the symbiotic units (host + algae)
- Algae exhibited rates of O₂ evolution lower than symbiotic units
- O₂ evolution increased to an intermediate level when crude HF was added back to algae
- All three fractions did not photoinhibit under 5- to 10-times saturating light at any temperature utilized

Photosynthesis of 28 m depth *Lubomirska baikalensis* sponge and isolated algae vs. of light intensity [Lake Baikal, Siberia, Russia]



Composition of 5 min photosynthetic products in isolated algae and colony of *Seriatopora coliendrum* vs. of habitat depths*

Sample	$\mu\text{g CO}_2 / 10^6\text{cells/h}$	Depth, m	Radioactivity of EtOH-water soluble fraction, % of total		
			Phosphate esters & sugars	Amino & organic acids	Glycerol & Gly-3-P
Algae (zooxanthellae)	10	3	65	19	16
	8	36	29	31	<u>40</u>
Corals		3	57	20	23
		36	25	25	<u>50</u>

*Indian Ocean, Praslin, Seychelles Islands

Influence of HF on photosynthesis of free living green algae *Chlamydomonas reinhardtii*



*HSM - high-salt minimal medium, pH 7.0

Modified from Gate et al 1995
Proc. Natl. Acad. Sci. USA 92:7430-7434

Applications

“Host Factor”:

1. May be applied for free living algae
2. Protects and stabilizes algae's pigments
3. Optimize photosynthesis, respiration, protein and first steps of lipid synthesis
4. Any amino acid rich medium, such as fish guts and other “waste-water” sources, may be considered as a good environment to enrich algae productivity

To optimize productivity:

Test “Host Factor” vs. salinity, temperature, light intensity, atmospheric pressure, anthropologic wastes, etc

On:

- algae growth rate;
- time of algae vegetation and division;
- quantitative and qualitative of products synthesis inside of the body and excreting into medium, etc.

Conclusions

- ❖ “Host Factor” optimizes environments for algae such that they fully realize their physiological potential
- ❖ Maximizing algae productivity will require conditions simulating “Host Factor” such as found in waste-water (this is in addition to optimal temperature, CO₂, light intensity, pH, etc)

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Dr. Stephen Gomez, Biomoda, Inc., Albuquerque, New Mexico, USA

Mr. Alexander Malkin, Bar Ilan University, Ramat Gan, Israel

Miss. Noa Eden, Bar Ilan University, Ramat Gan, Israel

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Many Thanks for Your Attention!